IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Applicant:	Rajiv SHAH, et al.
Title:	METHOD FOR FORMULATING A GLUCOSE OXIDASE ENZYME WITH A DESIRED PROPERTY OR PROPERTIES AND GLUCOSE OXIDASE ENZYME WITH THE DESIRED PROPERTY
Appl. No.:	10/035,918
Filing Date:	12/28/2001
Examiner:	Yong D. Pak
Art Unit:	1652
Confirmation No.:	2208

APPEAL BRIEF UNDER 37 C.F.R. § 41.37

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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This communication is an Appeal Brief, responsive to the Final Office Action dated June 27, 2008, concerning the above-referenced patent application.

Under the provisions of 37 C.F.R. § 41.37, this Appeal Brief is being filed with the appropriate appeal fee under 37 C.F.R. 41.20(b)(2). However, Applicant requests that the previously paid fees for filing two Appeal Briefs be applied to the present Appeal Brief. If that fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

More specifically, an appeal fee of $500.00 was submitted with Appliant's Appeal Brief dated December 7, 2005. Instead of filing an Answer in response to that Appeal Brief, the Examiner re-opened prosecution by issuing the Office Action dated March 8, 2006. In that

Office Action, the Examiner stated that the previously paid notice of appeal fee and appeal brief can be applied to a new appeal (Office Action dated March 8, 2006, pg. 2, ll. 9-10.)

Thereafter, Applicant filed a second Appeal Brief dated April 2, 2007, and requested that the previously paid fee for filing the first Appeal Brief dated December 7, 2005, be applied to that secondAppeal Brief. Instead of filing an Answer in response to that second Appeal Brief, the Examiner re-opened prosecution a second time by issuing the Office Action dated September 20, 2007. In that Office Action, the Examiner again stated that the previously paid notice of appeal fee and appeal brief can be applied to a new appeal (Office Action dated September 20, 2007, pg. 2.)

Accordingly, Applicant requests that the previously paid fees for filing the Appeal Brief dated December 7, 2005, and the Appeal Brief dated April 2, 2007, be applied to the present Appeal Brief. If that fee is deemed to be insufficient, authorization is hereby given to charge any deficiency (or credit any balance) to the undersigned deposit account 19-0741.

I. Real Party In Interest

The real party in interest for the above referenced patent application and the present Appeal is the assignee of record for the above referenced patent application, Medtronic Minimed, Inc., as recorded at Reel 012818, Frame 0025.

II. Related Appeals And Interferences

Except for the matters noted in this section, Applicant is not aware of any related appeals, interferences or legal proceedings that would have a bearing on the Board's decision in the present Appeal. However, Applicant filed a Notice of Appeal from a previous Final Office Action dated July 12, 2005, and filed a corresponding Appeal Brief on December 7, 2005. Following those filings, the Examiner reopened prosecution and issued a new Office Action. Thereafter, Applicant filed another Notice of Appeal, but from the Final Office Action dated September 20, 2007, and filed a corresponding Appeal Brief on March 20, 2008. Following those filings, the Examiner reopened prosecution a second time and issued a new Office Action. Thereafter, Applicant filed yet another Notice of Appeal and the present Appeal Brief, in response to the Final Office Action dated June 27, 2008.

In addition, co-pending continuation application no. 10/715,143 is presently pending and under rejection in a Final Office Action issued on August 7, 2008.

The present patent application claims the priority filing date of U.S. Provisional Application No. 60/335,585 (now expired), for which no substantive examination on the merits was conducted by the U.S. Patent and Trademark Office.

III. Status Of The Claims

Claims 1, 3-8, 10-46 and 48-54 are pending in the application.

However, claims 25-43 and 48-54 have been withdrawn from consideration by the Examiner.

Accordingly, claims 1, 3-8, 10-24 and 44-46 are pending and under consideration in the present application. Each of those claims is included in at least one of the rejections under general grounds identified in the Final Office Action and discussed in Sections VI. and VII., below.

The present appeal relates to each of the rejections and, thus, all of the rejected claims (i.e., claims 1, 3-8, 10-24 and 44-46).

IV. Status Of Amendments

No amendments have been filed, subsequent to the Final Office Action of June 27, 2008.

V. Summary Of Claimed Subject Matter

Embodiments of the present invention relate, generally, to a method employing directed evolution techniques for formulating a glucose oxidase enzyme having peroxide-resistant characteristics for use, by way of example, in a sensing device.

An example implantable sensing system contains a sensing device that is inserted into a vein, an artery, or any other part of a human body where it could sense a desired parameter of the implant environment. An enzyme may be placed inside of the sensing device and employed for sensing. If the device is a glucose-sensing device, then a combination of glucose oxidase (GOx)

and human serum albumin (HSA) may be utilized to form a sensor protein. During operation in a sensing device, glucose oxidase reacts with oxygen and oxidizes. The oxidation of glucose oxidase results in the formation of a hydroperoxy adduct, which transforms into hydrogen peroxide.

An obstacle to creating sensors that are long-lived and stable over time has been that glucose oxidase, when immobilized (e.g., for use in a sensor), undergoes oxidative inactivation by the aforementioned hydrogen peroxide over time. Since the lifetime of glucose sensors primarily depends on the lifetime of glucose oxidase, the effects of the peroxide on the glucose oxidase can severely limit the lifetimes of glucose sensors.

Prior processes for addressing the peroxide degradation of glucose oxidase have involved the use of additives or neutralizing agents for deactivating, removing or neutralizing peroxide. (Examples of such prior art are discussed below with respect to the Valdes et al. reference, the Stemmer patent, the Hatzinikolaou et al. article, the Wagner et al. patent, and the Aldrich Catalog page relating to Leuco-crystal violet) Embodiments of the present invention relate to a drastic change in direction of the state of the art by employing directed evolution techniques to formulate a glucose oxidase gene having desired peroxide resistant properties.

Evolution under non-stress circumstances takes years. Accordingly, evolution may be manipulated in embodiments of the invention for specific enzymatic functions. In embodiments of the invention, a technique known as directed evolution is employed to evolve glucose oxidase, to formulate a glucose oxidase that possesses improved resistance to peroxide. A glucose oxidase formulated pursuant to embodiments of the present invention may improve the longevity of a biosensor in which it is employed.

According to the claims under appeal, a method comprises creating a library of mutated glucose oxidase genes. Mutations of glucose oxidase genes may be obtained by performing polymerase chain reaction techniques, error-prone polymerase chain reaction techniques or gene shuffling techniques. Each of the library of mutants is inserted into a separate expression vector. Each expression vector is inserted into a host organism where a colony can grow, thereby replicating the mutated genes.

The library of colonies is then screened for desirable peroxide resistant properties. The colonies are screened by determining whether the colonies contain active glucose oxidase and determining whether the colonies have desired peroxide resistant properties. Determining whether the colonies have desired peroxide resistant properties involves incubating the colonies in peroxide and determining whether the colonies have active glucose oxidase after incubating, including measuring a concentration of the glucose oxidase.

In one embodiment, after the screening procedure, the glucose oxidase from one or more of the screened colonies may be mutated into a second generation library of mutants. The process may then proceed again with the second generation mutations. In other embodiments, this same process may be repeated many times on subsequent generations of mutated genes until a gene is formulated with suitable properties. In one embodiment the process is repeated from two to six times. In this manner, the mutations may be refined further to provide the desired peroxide resistant properties.

Those colonies that still contain active glucose oxidase after one or more mutation and incubation procedures may possess desirable peroxide resistant qualities. Glucose oxidase from those colonies still containing active glucose oxidase may be tested for functionality, for example, by immobilizing the glucose oxidase in a sensor. In other embodiments of the invention, following at least a portion of the screening procedure, the environments of the colonies may be altered another time if desired, for example, by adding more peroxide.

The method recited in the pending claims of the present application can provide significant advantages over the prior art of record. The ability to form a stable enzyme which is peroxide resistant and which may be employed in an altered environment (oxygen free environment), such as a biosensor, can provide significant advantages in extending the life of biosensors. When used in an implanted medical device (such as an implanted blood glucose sensor), peroxide resistance and, thus, a capability for extending the life of the enzyme can provide considerable patient comfort and safety advances, for example, by reducing the frequency of surgical sensor replacements. Moreover, the ability to form enzymes with peroxide resistant properties suitable for biosensor applications in a relatively inexpensive, non-complicated and reliable process can provide significant advantages with respect to the ability to

manufacture readily available supplies of the enzyme and, thus, increasing the availability of longer-life biosensors to more patients.

By a method in accordance with embodiments of the present invention, a glucose oxidase enzyme may be formulated to exhibit desired peroxide resistant properties. As such, further additives or other mechanisms for deactivating, removing or neutralizing peroxide may not be required. Thus, the disclosed method involves a distinct departure from the conventional direction of those skilled in the art.

Claim 1 is the sole independent claim under consideration in the present Appeal. An example of a mapping of claim 1 to the specification is shown in the following chart.

Claim 1	Specification
A method for formulating an enzyme comprising:	Title; pg. 1, ll. 21-24; pg. 4, ll. 10-22; pg. 7, ll. 11-12.
obtaining a library of glucose oxidase genes;	Pg. 5, ll. 14-16; pg. 8, ll. 6-13; Fig. 2, ref. 12; pg. 14, ll. 1-8.
creating a library of mutated glucose oxidase genes;	Pg. 5, ll. 15-16; pg. 8, l. 14 to pg. 9, l. 9; Fig. 5; pg. 14, ll. 9-14; Fig. 2, ref. 14.
introducing each mutated glucose oxidase gene of the library into separate expression vectors;	Pg. 5, l. 16; pg. 9, ll. 10-16; Fig. 2, ref. 16.
inserting the expression vectors into non-human host organisms;	Pg. 5, l. 17; pg. 9, ll. 17-22; Fig. 2, ref. 16.
growing colonies of the host organisms; and	Pg. 5, l. 17-18; pg 10, ll. 1-9; Fig. 2, ref. 18.
screening the colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase and determining	Pg. 5, ll. 18-21; pg. 10, l. 10 to pg. 13, l. 15; pg. 14, ll. 15-20; Fig. 2, ref. 20.

Claim 1	Specification
whether the colonies have predefined, desired peroxide resistant properties,	
wherein determining whether the colonies have predefined, desired peroxide resistant properties comprises: incubating the colonies in peroxide; and determining whether the colonies have active glucose oxidase after incubating the colonies in peroxide.	Pg. 11, ll. 15-22; pg. 12, ll. 5-7

VI. Grounds Of Rejection To Be Reviewed On Appeal

Claims 1, 3-8, 10-24 and 44-46 are rejected, as follows:

1. Claims 1, 3-5, 12-15, 18-24 and 44-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Cherry et al., and Hatzinikolaou et al.

2. Claims 16 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Cherry et al., and Hatzinikolaou et al. and further in view of MIXONIX.

3. Claims 6-8, 10-11 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Stemmer (apparently intended to state Cherry et al.), and Hatzinikolaou et al. and further in view of Wagner. However, the Examiner's arguments also appear to further combine the Aldrich Catalog reference with the above-listed four references.

As noted in Section III, above, the present appeal relates to each of the above rejections and, thus, all of the rejected claims (i.e., claims 1, 3-8, 10-24 and 44-46).

VII. Argument

1. Appeal Of Rejection Of Claims 1, 3-5, 12-15, 18-24 and 44-45 are rejected under 35 U.S.C. 103(a)

Claims 1, 3-5, 12-15, 18-24 and 44-45 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Cherry et al. and Hatzinikolaou et al. This rejection is respectfully traversed and reversal of the rejection is requested.

Claim 1 recites a method for formulating an enzyme that is not disclosed by either Valdes et al., Cherry et al. or Hatzinikolaou et al., alone or in the combination suggested by the Examiner (which combination is traversed as discussed below). For example, the method of claim 1 recites, among other features:

"creating a library of mutated glucose oxidase genes,"

"growing colonies of the host organisms," and

"screening the colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase and determining whether the colonies have predefined, desired peroxide resistant properties, wherein determining whether the colonies have predefined, desired peroxide resistant properties comprises: incubating the colonies in peroxide; and determining whether the colonies have active glucose oxidase after incubating the colonies in peroxide."

The method recited in claim 1 recites several actions that, together, form the claimed method, where none of the above-cited references describes the complete method of all of the actions recited in claim 1. In forming the rejection, the Examiner has selected and chosen certain pieces of various discrete processes from each of the above-cited references and formed a mosaic of those pieces in an attempt to arrive at the present invention. The cited references provide no suggestion or motivation for the selecting pieces of the disclosed processes and combine them in the fashion that the Examiner suggests. Instead, the references, themselves, as well as other references of record teach a direction away from the present invention.

The mass of evidence of record in the application suggests that those skilled in the art were taking a direction that was completely different from that of the claimed invention. While the Examiner raises arguments as to obviousness to combine various parts of the cited references,

none of the evidence of record supports the Examiner's proposal to select and combination portions of the various references. To the contrary, a number of references of record (including the primary reference relied upon by the Examiner) teach a direction different than the claimed invention and would lead one of ordinary skill in the art away from the claimed invention. Without the present disclosure as a guide, one of ordinary skill in the art would not have found it obvious to combine the above-cited references as suggested by the Examiner.

As described in more detail below:

a. The prior art of record does not teach or suggest the claimed invention;

b. None of the prior art of record provides any teaching or suggestion for the combination of the Valdes et al, Cherry et al., and Hatzinikolaou et al., and the mass of evidence of record shows that the prior art teaches away from the claimed invention;

c. Each of dependent claims 3-5, 10-15, 18 and 20-24 recite further features that distinguish those claims from the prior art.

a. The Rejection Is Improper Because The Prior Art Does Not Teach Or Suggest The Claimed Invention.

In particular, neither Valdes et al. nor Cherry et al., nor Hatzinikolaou et al. describe formulating a glucose oxidase enzyme by mutating glucose oxidases to make them resistant to peroxide degradation. Moreover, one of ordinary skill in the art would not have been led by the prior art of record to mutate glucose oxidase genes, much less to mutate such genes and screen for desired peroxide resistance properties. Such procedures would have been a drastic departure from the state of the art and, without the benefit of the present specification as a guide, would not have been obvious to one of ordinary skill in the art.

The Examiner argues that Valdes et al. teaches that glucose oxidases in glucose sensors degrade over time due to hydrogen peroxide. (Office Action of September 20, 2007, pg. 5, ll. 12-14.) The Examiner acknowledges that Valdes et al. do not teach a method of producing mutant glucose oxidase that is resistant to degradation from peroxide. (Office Action of June 27, 2008,

pg. 4, ll. 15-17; Office Action of September 20, 2007, pg. 6, ll. 3-5 and Final Office Aciton of November 1, 2006, pg. 5, ll. 3-6.) As discussed in more detail below, instead, Valdes et al. teach to address peroxide degredation by adding a chemical catalase or by attaching an immobilized enzyme to a support that deactivates hydrogen peroxide.

The Examiner argues that Valdes et al. teach to "prevent the degredation of the enzyme" instead of replacing the enzyme, citing Valdes et al. at page 375. (Office Action of June 27, 2008, pg. 4, ll. 6-9; Office Action of September 20, 2007, pg. 5, ll. 15-17.) The Examiner quoted a portion of Valdes et al.'s statement in a manner that takes it out of context, as a springboard to imply that Valdes et al. would have suggested a process involving creating mutated genes of glucose oxidase and screening colonies in the manner recited in the present claims. However, Valdes et al. immediately follow that statement with a description of the use of chemical addititives as the so-called "better options." Accordingly, Valdes et al. teach a specific direction (use of chemical additives) that departs from the then-conventional process of replacing a degraded enzyme with a fresh enzyme.

Valdes et al. is not the only reference of record that teaches that the direction taken by those skilled in the art was to use chemical additives. Indeed, other references of record similary teach that direction of the art (e.g., U.S. Patent No. 6,689,265 to Heller et al. and the article titled "Glucose ENFET doped with MnO_2 powder" by Yin et al, attached as Exhibits 1 and 2, respetively). Neither Valdes et al., nor any prior art of record that relates to peroxide degredation of glucose oxidase, describe or suggest the use of a gene mutation method for addressing peroxide degredation of glucose oxidase. Instead, as described in more detail below, Valdes et al. and other references of record show that the direction taken by those skilled in the art was away from the method of the presently claimed invention.

Because of this lack of disclosure in Valdes et al., the Examiner attempts to select pieces of each of the Cherry et al. and Hatzinikolaou et al. references. However, none of those references teach formulating a glucose oxidase enzyme by mutating glucose oxidases to make them resistant to peroxide degradation.

For example, as noted above, claim 1 recites a method for formulating an enzyme that includes, among other features, "obtaining a library of glucose oxidase genes" and "creating a library of mutated glucose oxidase genes."

The Examiner acknowledges that Valdes et al. do not teach a method of producing mutant glucose oxidase that is resistant to degradation from peroxide. (Office Action of June 27, 2008, pg. 4, ll. 15-17; Office Action of September 20, 2007, pg. 6, ll. 3-5.) Indeed, Valdes et al. do not teach of producing a mutant of any enzyme for any purpose. Accordingly, Valdes et al. do not teach or suggest creating a library of mutated glucose oxidase genes.

The Examiner cited Cherry et al. as disclosing a method of making mutants of an enzyme which is degraded in the presence of hydrogen peroxide, by using directed evolution techniques. (Office Action of June 27, 2008, pg. 4, l. 21 to pg. 5, l. 1; Office Action of September 20, 2007, pg. 6, ll. 9-11.) The Examiner states:

> "Cherry et al. discloses that after multiple rounds of directed evolution an enzyme, mutants of said enzyme that are resistant to deactivation in the presence of high concentration of hydrogen peroxide, conditions that mimic of hydrogen peroxide wherein the enzyme is normally deactivated, were obtained (pages 380-382). Cherry et al. discloses that colonies having enzymatic activity were selected to determine for its resistance against hydrogen peroxide (page 382)." (Office Action of June 27, 2008, pg. 5, ll. 1-5; Office Action of September 20, 2007, pg. 6, ll. 9-17.)

However, Cherry et al., like Valdes et al., fail to disclose or suggest "creating a library of mutated glucose oxidase genes." Cherry et al. have nothing to do with glucose oxidase genes and would not teach or suggest mutating glucose oxidase genes. Instead, Cherry et al. describe production of a detergent additive that is able to catalyze the oxidation of dyes that leach out of colored clothing during a wash cycle to render the dyes colorless and effectively prevent the transfer of dye to other clothes. (Cherry, et al., pg. 379, col. 1, ll. 24-29.)

According to Cherry, et al., "[i]n wash conditions using bleach-containing detergents, the elevated pH and high peroxide concentrations favor rapid formation of inactive form of the enzyme." Cherry et al. state that the "goal" of their work "was to develop a peroxidase variant effective as a dye-bleaching reagent in detergent." (Cherry et al., pg. 379, col. 2, ll. 7-11.)

Cherry et al. teaches to produce a dye-bleaching reagent in a clothes washing detergent and do not teach or suggest anything to do with mutating glucose oxidase genes. The Examiner's apparent attempt to rely on Cherry et al. as teaching of directed evoloution of enzymes, in general, to be resistant to peroxide takes Cherry et al.'s disclosure far out of context. The only reason that Cherry et al. is concerned about peroxide resistance is because Cherry et al.'s stated goal was to develop a dye-bleaching reagent in a clothes washing detergent (where "wash conditions" have "high peroxide conentrations"). Cherry et al.'s "inactivation conditions were designed to mimic those found in washing machines using bleach containing commercial detergents," at pH levels and temperatures ("40-50 C") inconsistent with the production of glucose oxidase. (Cherry et al., pg. 380, col. 1, ll. 19-21.) Cherry et al.'s reference to peroxide resistance and inactivation conditions would not teach or suggest anything to one skilled in the art with regard to "creating a library of mutated glucose oxidase genes."

The context in which Cherry et al. refer to peroxide resistance (clothes washing environments) would not have been ignored by one of ordinary skill in the art. Such contexts (and environments) are inconsistent with the production of glucose oxidase. Thus, like Valdes et al., Cherry et al. fail to disclose or suggest "creating a library of mutated glucose oxidase genes."

The Examiner stated that Hatzinikolaou et al. discloses a library of glucose oxidase genes known in the art, a method of isolating and purifying glucose oxidase and methods of measuring glucose oxidase activity and concentration of glucose oxidase. (Office Action of June 27, 2008, pg. 5, ll. 8-12; Office Action of September 20, 2007, pg. 6, ll. 18-20.) However, Hatzinikalaou et al. describe isolating and characterizing a new synthesized glucose oxidase for purposes of conducting certain specified analyses (described on pages 373 and 374 of the Hatzinikalaou et al. reference), none of which relate to resistance to hydrogen peroxide.

While gene libraries have been employed by those skilled in the art for gene analysis, Hatzinikolaou et al. provide no suggestion to use such libraries in the formulation of an ezyme by directed evolution. Hatzinikolaou et al. teaching of using gene libraries to analyze characterisitics of a gene provides no motivation or suggestion to do anything more than to analyze the specific new synthesized glucose oxidase for the specific characteristics described on pages 373 and 374 of that reference. The Examiner has picked only the feature of forming a gene

library of glucose oxidase gene from Hatzinikolaou et al.'s overall process and seeks to combine that teaching with Cherry et al. and Valdes et al.

However, Hatzinikalaou et al.'s purpose of forming a library of a particular new simulated glucose oxidase (for analyzing the characterisitics of the new simulated glucose oxidase described in that reference) would have no applicable purpose in any mutation process described by Cherry et al. Once Hatzinikolaou et al. obtains and isolates a sample of the new glucose osidase, Hatzinikolaou et al. conducts analsyis on the isolated sample. Mutating the sample would not allow Hatzinikalaou et al. to analyze the characteristics of the simulated glucose oxidase (as the mutations could effect the detection of characteristics under analsyis). Accordingly, it would not have been obvious to look to Hatzinikolaou et al. as a teaching of obtaining a library of glucose oxidase genes that are to be mutated to create a library of mutated glucose oxidase genes. The Examiner's suggestion to combine Hatzinikolaou et al. with Cherry et al. and Valdes et al. is, therefore, respectfully traversed. Moreover, Hatizinikolaou et al. does not disclose or suggest screening colonies for active glucose oxidase predefined, desired peroxide resistant properties.

Claim 1 of the present application further recites: "screening the colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase and determining whether the colonies have predefined, desired peroxide resistant properties." Claim 1 further recties that "determining whether the colonies have predefined, desired peroxide resistant properties comprises: incubating the colonies in peroxide; and determining whether the colonies have active glucose oxidase after incubating the colonies in peroxide."

Because Cherry et al. do not relate to obtaining a library of glucose oxidase genes or a library of mutated glucose oxidase genes, it follows that Cherry et al. also do not describe screening colonies by determining whether the colonies contain active glucose oxidase and determining whether the colonies have predefined, desired peroxide resistenat properties. Also, Hatzinikolaou et al. provide no teaching or suggestion of screening colonies for active glucose oxidase having a desired peroxide resistant property. While the combination of those references is traversed for reasons noted herein, no combination of those references could lead to screening colonies for active glucose oxidase having a desired peroxide resistant property because neither

of those references, alone, describe such a feature. Moreover, as noted above, because Valdes et al. also do not teach to screening colonies for active glucose oxidase having a desired peroxide resistant property and, instead, teach a very different direction (addition of chemicals to reduce peroxide degredation), the Examiner's suggestion to combine Valdes et al. with Cherry et al. and Hatzinikolaou et al. is traversed and still would not lead to the present invention. Accordingly, reversal of the rejection based on that improper combination is requested.

None of the cited Valdes et al., Cherry et al. and Hatzinikolaou et al. references describes or suggests creating a library of mutated glucose oxidase genes and then screening colonies for active glucose oxidase having a desired peroxide resistant property. Accordingly, the combination of the references (as suggested by the Examiner) could not result in the claimed invention. The rejection of claims 1, 3-5, 12-15, 18-24 and 44-45 under 35 U.S.C. 103(a) is, therefore, respectfully traversed and should be reversed.

b. The Rejection Is Improper Because Prior Art Provides No Motivation To Combine And Teaches Away From The Combination Suggested By The Examiner.

Because of the above-noted lack of disclosure in Valdes et al. (of creating a library of mutated glucose oxidase genes and screening colonies for active glucose oxidase predefined, desired peroxide resistant properties), the Examiner attempts to select pieces of each of the Cherry et al. and Hatzinikolaou et al. reference out of context and combine them with the Valdes et al. reference. The present rejection is the third attempt by the Examiner to combine the Valdes et al. reference with other references.

An attempt to combine the Valdes et al. reference with other references of record (i.e., article titled "Current Protocols in Molecular Biololgy") was previously made in the rejections raised in the Final Office Action of July 12, 2005. In Applicant's Appeal Brief filed on December 7, 2005, Applicant explained that the combination was improper because of the direction of teaching of Valdes et al. (of using chemical additives) would have led one of ordinary skill in the art away from gene mutation methods taught by the article titled "Current Protocols in Molecular Biololgy." In response to Applicant's Appeal Brief of December 7, 2005, the Examiner re-opened prosecution and apparently withdrew previous rejections over prior art

(as those previous rejections were not applied), but raised a new ground of rejection by attempting to combine the Valdes et al. reference with Stemmer, Hatzinikolaou et al., Wagner et al. and Aldrich Catalog.

After defending the claims over that new rejection, Applicant filed a second Appeal Brief on April 2, 2007, in which Applicant explained that the combination of the Valdes et al. reference with Stemmer, Hatzinikolaou et al., Wagner et al. and Aldrich Catalog also was improper because of the direction of teaching of Valdes et al. (of using chemical additives) would have led one of ordinary skill in the art away from gene mutation methods taught by the Stemmer. In response to that Appeal Brief, the Examiner again re-opened prosecution and apparently withdrew previous rejections over prior art (as those previous rejections were not applied), but raised yet a new ground of rejection by yet again attempting to combine Valdes et al. with other references (in particular to combine the Valdes et al. reference with Cherry et al. and Hatzinikolaou et al.

Neither the Cherry et al. reference nor the Hatzinikolaou et al. reference provide any teaching of creating a library of mutated glucose oxidase genes and screening colonies for active glucose oxidase with predefined, desired peroxide resistant properties, or methods of formulating a glucose oxidase enzyme with peroxide resistance. Accordingly, each of those references is no more relevant to those features of the claimed invention as was the article titled "Current Protocols in Molecular Biololgy" or the Stemmer patent.

In addition to the inconsistent issuance of a new rejection based on an attempt to combine Valdes et al. with other references relating to gene mutation (after the combination of Valdes et al. with the article titled "Current Protocols in Molecular Biololgy" was traversed and apparently overcome and the combination of Valdes et al. and Stemmer, Hatzinikolaou et al and other references was traversed and apparently overcome), the new rejections under 35 U.S.C. 103(a) are improper because of a lack of motivation (without the present disclosure as a guide) to pick and choose various discrete features out of mutliple references and combine those features as suggested by the Examiner.

The Examiner cites no teaching in either the Valdes et al. reference, the Cherry et al. reference or the Hatzinikolaou et al. reference (or any other prior art) for incubating mutated colonies of glucose oxidase with hydrogen peroxide. In fact, none of the Valdes et al., Cherry et al. or Hatzinikolaou et al. references provide any motivation or suggestion for creating a library of mutated glucose oxidase genes and screening colonies for active glucose oxidase and desired peroxide resistant properties. Indeed, Valdes et al. teach away from such methods by, instead, referring to conventional procedures (using additives for deactivating or destroying hydrogen peroxide and, thus, teach away from such a method, as follows:

> "To prohibit the H_2O_2 from degrading the GOD enzyme, it has been proposed that catalase be coimmobilized with GOD ... The addition of catalase in either the GOD itself, or to the incubating solution has resulted in a slower deactivation of the GOD enzyme ... A long term remedy of the degradation of GOD by H_2O_2 could be the immobilization and attachment of the enzyme to a support that deactivates H_2O_2, as it is being produced. Such as study was conducted by Cho[2], using the peroxide decomposition catalyst, activated carbon. In a study conducted by Carter[19], the best results were obtained with activated carbon, impregnated with ruthenium. This combination was able to destroy hydrogen peroxide and stabilized the enzyme." (Valdes et al., pg. 375, col. 1, l.18 to col. 2, l. 6.)

Not only does Valdes et al. fail to teach or suggest to mutate glucose oxidase and screen mutated glucose oxidase for peroxide resistance properties, but, in the above-quoted statement, Valdes et al. further teaches to use other, very different procedures (conventional in the art) to address degradation effects of peroxide on glucose oxidase. Thus, the Valdes et al. reference shows that the direction taken by those most skilled in the art involved employing materials, additives, or the like that deactivate peroxide.

Additional prior art of record also describes conventional "additive" processes for removing or neutralizing peroxide such as by adding an antioxidant or peroxidase to the glucose oxidase to break down peroxide or by coating the glucose oxidase enzyme with a protective coating, including U.S. Patent No. 6,689,265 to Heller et al. (Exhibit 1) and the article titled "Glucose ENFET doped with MnO_2 powder" by Yin et al (Exhibit 2). Those prior art references further emphasize that the direction taken by those skilled in the art for addressing the peroxide degradation of glucose oxidase is wholly different from the direction of the present invention. In U.S. Patent No. 6,689,265 to Heller et al., a peroxide generating enzyme may include a

sufficiently thick, natural, electrically insulating protein or glycoprotein layer. (See column 6, lines 59-67 of the Heller et al. patent, Exhibit 1.) Heller et al. also disclose an alternative embodiment in which a peroxide generating enzyme is immobilized in a non-conducting inorganic or organic polymeric matrix. (See column 7, lines 3-11 of the Heller et al. patent, Exhibit 1.) Also, Heller et al. describe a first layer enzyme 11 (peroxidase) that reduces peroxide generated from a second layer (glucose oxidase layer) 13. The Yin et al. article describes the addition of MnO_2 to catalyze peroxide and produce water and oxygen therefrom. (Yin, Exhibit 2, Abstract and pg. 188, col. 1, ll. 20-34.)

Thus, both the Heller et al. patent and the Yin et al. article show that the direction taken by those skilled in the art is to provide additives or complex multi-layer sensor structures to remove hydrogen peroxide. These references, in addition to Valdes et al.'s express references to conventional uses of additives, show that those skilled in the art were not considering mutating glucose oxidase genes and growing and screening colonies for peroxide resistance, but instead were attempting to address the peroxide production issue by removing or neutralizing peroxide with additives (not by altering the glucose oxidase). The state and direction of the prior art, as evidenced by Valdes et al., Heller et al. and Yin et al., was a wholly different direction than that taken by the present Applicants (including creating a library of mutated glucose oxidase enzyme genes and screening colonies for desirable properties by determining whether the colonies contain active glucose oxidase and determining whether the colonies have desired peroxide resistant properties.) Accordingly, the mass of evidence of record (including the primary reference relied upon by the Examiner) teaches one skilled in the art in a direction different from (and away from) the present invention.

Without the present disclosure as a guide, one of ordinary skill in the art would not have found Valdes et al.'s discussion of the degradation of glucose oxidase as a prompt or suggestion to employ a mutation process as described Cherry et al. Instead, as noted above, one of ordinary skill in the art would have looked to conventional manners of removing peroxide, such as additives for removing or neutralizing peroxide. Accordingly, the rejection of 1, 3-5, 12-15, 18-24 and 44-45 under 35 U.S.C. 103(a) is further respectfully traversed and reversal of the rejection is requested.

The fact that the primary reference (Valdes et al.) teach away from the clamed invention and the combination suggested by the Examiner, shows that a *prima facie* case of obviousness has not been raised. Numerous Federal Circuit decisions recognize that an invention will not be deemed obvious in a patent law sense when one or more prior art references "teach away" from the invention. For example, the Federal Circuit stated "as a useful general rule, that references that teach away cannot serve to create a prima facie case of obviousness." *McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1354, 60 USPQ2d 1001 (Fed. Cir. 2001).

Furthermore, "an applicant may rebut a *prima facie* case of obviousness by showing that the prior art teaches away from the claimed invention in any material respect." *In re Peterson*, 315 F.3d 1325, 1331, 65 USPQ2d 1379 (Fed. Cir. 2003). Also see, *Gillette Co. v. S.C. Johnson & Sons, Inc.*, 919 F.2d 720, 724, 16 USPQ2d 1923, 1927 (Fed. Cir. 1990)(the closest prior art reference "would likely discourage the art worker from attempting the substitution suggested by [the inventor/patentee]") and *Singh v. Brake*, 317 F.3d 1334, 1346, 65 USPQ2d 1641 (Fed. Cir. 2003)("whether or not a reference 'teaches away' from a claimed invention" is "relevant in determining whether or not a claimed invention would have been obvious").

When patentability turns on the question of obviousness, the search for and analysis of the prior art includes evidence relevant to the finding of whether there is a teaching, motivation, or suggestion to select and combine the references relied on as evidence of obviousness. (underline added for emphasis.) *See, e.g., McGinley v. Franklin Sports, Inc.*, 262 F.3d 1339, 1351—52, 60 USPQ2d 1001, 1008 (Fed. Cir. 2001) ("the central question is whether there is reason to combine [the] references," a question of fact drawing on the *Graham* factors).

Conclusory statements that prior art references provide motivation to combine, or statements of motivation derived from the Applicant's own specification, are not sufficient to set forth a *prima facie* case of obviousness. "The factual inquiry whether to combine references must be thorough and searching." *Id.* It must be based on objective evidence of record. This precedent has been reinforced in myriad decisions. *See, e.g., Brown & Williamson Tobacco Corp. v. Philip Morris Inc.*, 229 F.3d 1120, 1124— 25, 56 USPQ2d 1456, 1459 (Fed. Cir. 2000) ("a showing of a suggestion, teaching, or motivation to combine the prior art references is an 'essential component of an obviousness holding'") (quoting *C.R. Bard. Inc., v. M3* Systems, *Inc.*,

157 F.3d 1340, 1352, 48 USPQ2d 1225, 1232 (Fed. Cir. 1998)); *In re Dembiczak,* 175 F.3d 994, 999, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("Our case law makes clear that the best defense against the subtle but powerful attraction of a hindsight-based obviousness analysis is rigorous application of the requirement for a showing of the teaching or motivation to combine prior art references."); *In re Dance,* 160 F.3d 1339, 1343, 48 USPQ2d 1635, 1637 (Fed. Cir. 1998) (there must be some motivation, suggestion, or teaching of the desirability of making the specific combination that was made by the applicant); *In re Fine,* 837 F.2d 1071, 1075, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988) ("'teachings of references can be combined *only* if there is some suggestion or incentive to do so.') (emphasis in original) (quoting *ACS Hosp.* Sys., *Inc. 'v. Montefiore Hosp.,* 732 F.2d 1572, 1577, 221 USPQ 929, 933 (Fed. Cir. 1984)).

As noted above, the Examiner has not shown any motivation or suggestion in the prior art that would have led one skilled in the art to select a mutation and screening process described by the Cherry et al. for creating a library of mutated glucose oxidase and screening colonies for peroxide resistant properties. In fact, Valdes et al and other prior art of record show that a selection of a mutation and screening process would have been a drastic diversion from the direction taken by those most skilled in the prior art.

The legal authority expresses the requirement for a showing of specificity in the prior art of motivation to select components to combine. *See, e.g., In re Kotzab,* 217 F.3d 1365, 1371, 55 USPQ2d 1313, 1317 (Fed. Cir. 2000) ("particular findings must be made as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed"); *In re Rouffet,* 149 F.3d 1350, 1359, 47 USPQ2d 1453, 1459 (Fed. Cir. 1998) ("even when the level of skill in the art is high, the Board must identify specifically the principle, known to one of ordinary skill, that suggests the claimed combination. In other words, the Board must explain the reasons one of ordinary skill in the art would have been motivated to select the references and to combine them to render the claimed invention obvious."); *In re Fritch,* 972 F.2d 1260, 1265, 23 USPQ2d 1780, 1783 (Fed. Cir. 1992) (the examiner can satisfy the burden of showing obviousness of the combination "only by showing some objective teaching in the prior art or that knowledge generally available to one of ordinary skill in the art would lead that individual to combine the relevant teachings of the references").

While the Valdes et al., Cherry et al. and Hatzinikolaou et al. references, themselves, provide no motivation or suggestion, the Examiner argues that one of ordinary skill in the art would have been motivated to do so in order to generate active glucose oxidase that is resistant to peroxide, to use them in glucose sensors and prolong their use. (Office Action of June 27, 2008, at page 6, lines 1-3 and page 9, line 21 to page 10, line 2; Office Action of September 20, 2007, at pg. 7, ll. 1-13 and Final Office Action dated November 1, 2006, pg. 7, ll. 4-7.) While this is the likely conclusion, after reading the present disclosure as a guide, the references of record actually teach to do something very different (add chemicals) to reduce peroxide degradation of glucose oxidase. Thus, the mass of evidence of record shows that the motivation provided by the cited references would have been to reduce peroxide degredation by adding chemicals as taught by Valdes et al., U.S. Patent No. 6,689,265 to Heller et al. (Exhibit 1) and the article titled "Glucose ENFET doped with MnO_2 powder" by Yin et al (Exhibit 2).

The Examiner also argues that motivation to combine the cited references comes from the combined teachings of the cited references. (Office Action of June 27, 2008, at page 10, lines 16-18.) However, as noted above, the references of record teach a different direction for addressing peroxide degredation than that taken by the claimed invention. Thus, if any motivation would have been derived from such references, it would have been to reduce peroxide degredation by adding chemicals as taught by Valdes et al., U.S. Patent No. 6,689,265 to Heller et al. (Exhibit 1) and the article titled "Glucose ENFET doped with MnO_2 powder" by Yin et al (Exhibit 2).

Without the present disclosure as a guide, one of ordinary skill in the art would not have selected gene mutation processes for producing dye-bleaching reagents for detergents, and glucose oxidase purifying, isolating and measuring processes to modify Valdes et al.'s disclosed solution to peroxide degradation of glucose oxidase. Valdes et al. teaches solutions to the peroxide degredation problem (by using chemical additives) and would have led one skilled in the art in the direction of those solutions. Cherry et al. do not mention glucose oxidase anywhere in its disclosure. Hatzinikolaou et al. fails to provide any motivation or suggestion of any relation to a gene mutation procedure or of addressing peroxide degradation of glucose oxidase.

Moreover, the whole purpose of Hatzinikolaou et al. (to analyze a specific new similated glucose oxidase) is not consistent with Cherry et al.'s mutation methods.

The Examiner's conclusory statements of suggestion to combine fail to address the significant issue of why one skilled in the art would have been motivated to select a process as described by Cherry et al., to change the direction taken by those most skilled in the prior art as described by Valdes et al. The prior art teaches that those most skilled in the art were taking a wholly different direction to address peroxide degradation of glucose oxidase and, thus, would have found it unreasonable to change the course of direction from that of the state of the art.

More specifically, Valdes et al. refer to completely different directions taken by those most skilled in the art, whereby the glucose oxidase enzyme is immobilized and attached to a support that deactivates peroxide. "A reference may be said to teach away when a person of ordinary skill, upon reading the reference, ... would be led in a direction divergent from the path that was taken by the applicant." *Tec Air, Inc. v. Denso Mfg. Mich. Inc.*, 192 F.3d 1353, 1360, 52 USPQ2d 1294, 1298 (Fed. Cir. 1999). Valdes et al., directly refers the reader to conventional methods of addressing peroxide degradation of glucose oxidase that employ additives for destroying or neutralizing peroxide (which is quite different from creating a library of mutated genes and screening for desired peroxide resistant properties).

Because the Examiner has not shown any motivation or suggestion in the prior art that would have led one skilled in the art to select Cherry et al.'s mutation process with the Valdes et al. reference, the Examiner has not raised a *prima facie* case of obviousness. Therefore, the rejection of 1, 3-5, 12-15, 18-24 and 44-45 under 35 U.S.C. 103(a) is respectfully traversed and reversal of the rejection is requested.

c. Each of dependent claims 3-5, 10-15, 18 and 20-24 recite further features that distinguish those claims from the prior art.

Each of dependent claims 3-5, 10-15, 18, and 20-24 recite further features that distinguish those claims from the prior art. In particular, each of those claims recites features relating to colonies of mutated glucose oxidase gene. As described above, neither Valdes et al. nor the Cherry et al., Hatzinikolaou et al, MISONIX, Wagner et al or Aldrich Catalog references, alone

or in combination, describe or suggest mutating glucose oxidase and screening for active glucose oxidase and predefined, desired peroxide resistant properties. In that regard, those references also do not disclose or suggest the additional processing recited in dependent claims 3-5, 10-15, 18, and 20-24, including:

1. "screening the colonies for desirable properties further comprises testing glucose oxidase from the colonies for functionality" (claim 3);

2. "determining whether the colonies have peroxide resistant properties is only performed if results of determining whether the colonies contain active glucose oxidase are positive" (claim 4);

3. "testing glucose oxidase from the colonies for functionality is only performed if results of determining whether the colonies contain active glucose oxidase are positive and if results of determining whether the colonies have peroxide resistant properties are positive" (claim 5);

4. "testing glucose oxidase from the colonies for functionality comprises employing glucose oxidase from the colonies in sensors" (claim 10);

5. "extracting glucose oxidase from the colonies; immobilizing the glucose oxidase after extracting the glucose oxidase from the colonies; placing the immobilized glucose oxidase in a sensor; and testing the sensor for functionality in a test environment" (claim 11);

6. "employing an ionic column to extract glucose oxidase from the colonies" (claim 12);

7. "removing the glucose oxidase from the colonies; purifying the glucose oxidase; and characterizing the glucose oxidase" (claim 13);

8. "grinding the colonies in a homogenizer into cell components" (claim 14);

9. "fractionating the cell components employing centrifugation and differential solubility after grinding the colonies in a homogenizer" (claim 15);

10. "purifying the glucose oxidase by employing chromatography methods" (claim 18).

11. "creating at least one mutated glucose oxidase gene comprises employing polymerase chain reaction techniques to create at least one mutated glucose oxidase gene" (claim 20);

12. "employing error-prone polymerase chain reaction techniques to create at least one mutated glucose oxidase gene" (claim 21);

13. "employing gene shuffling techniques to create at least one mutated glucose oxidase gene" (claim 22);

14. "creating a next generation of mutated glucose oxidase genes after screening the colonies for desirable properties" (claim 23); and

15. "creating a next generation of mutated glucose oxidase genes is repeated approximately 2 to 6 times" (claim 24).

The rejection of claims 3-5, 8, 10-15, 18 and 20-24 is, therefore further respectfully traversed and reversal of the rejection is requested.

2. Appeal Of Rejection Of Claims 16 And 17 Under 35 U.S.C. 103(a)

Claims 16 and 17 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Cherry et al. and Hatzinikolaou et al. and further in view of MISONIX.

This rejection is respectfully traversed at least for reason discussed above with respect to claim 1. Each of claims 16 and 17 is indirectly dependent on claim 1. Accordingly, the distinctions noted above between claim1 and the cited Valdes et al., Cherry et al. and Hatzinikolaou et al. references apply to claims 16 and 17, as well. The MISONIX reference was not relied upon by the Examiner to address those distinctions. Instead, the Examiner cited the MISONIX reference as allegedly teaching of disrupting cells via sonication.

Accordingly, at least for reasons discussed above with respect to claim 1, the rejection of claims 16 and 17 is respectfully traversed and reversal of the rejection is requested.

3. Appeal Of Rejection Of Claims 6-8, 10-11 and 46 Under 35 U.S.C. 103(a)

Claims 6-8, 10, 11 and 46 are rejected under 35 U.S.C. 103(a) as being unpatentable over Valdes et al., Stemmer et al. (sic, apparently Cherry et al.) and Hatzinikolaou et al. and further in view of Wagner.

This rejection is respectfully traversed at least for reason discussed above with respect to claim 1. Each of claims 6-8, 10, 11 and 46 is directly or indirectly dependent on claim 1. Accordingly, the distinctions noted above between claim1 and the cited Valdes et al., Cherry et al. and Hatzinikolaou et al. references apply to claims 6-8, 10, 11 and 46, as well. The Wagner reference was not relied upon by the Examiner to address those distinctions. The Wagner reference does not address the above-noted distinctions between the claims and the Valdes et al., Cherry et al. and Hatzinikolaou references.

Indeed, the Wagner reference was cited, according to the Examiner, for disclosing a method of determining glucose oxidase activity via a sensor by measuring fluorescence emission from a dye, wherein oxidation of glucose by active glucose oxidase reduces the flurescence emission. However, Wagner does not teach or suggest formulating a glucose oxidase enzyme by mutating glucose oxidases to make them resistant to peroxide degradation. Accordingly, the combination of Wagner with the above-discussed references (the Valdes et al., Cherry et al. and Hatzinikolaou references) would not lead to the presently claimed invention.

The Examiner also cited the Aldrich Catalog as describing Leuco-crystal violet dyes as common fluouresent dyes. The cited portion of the Aldrich Catalog neither describes nor suggests formulating an enzyme, much less creating a library of mutated glucose oxidase genes, screening colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase or determining whether the colonies have predefined desired peroxide resistant properties. Accordingly, the cited portion of the Aldrich Catalog does not address the above-noted distinctions between the claimed invention and the Valdes et al., Cherry et al., Hatzinikolaou et al. and Wagner references. Thus, the combination of the cited portion of the Aldrich Catalog with those other references (as suggested by the Examiner) could not result in the claimed invention.

Accordingly, at least for reasons discussed above with respect to claim 1, the rejection of claims 6-8, 10, 11 and 46 is respectfully traversed and reversal of the rejection is requested.

VIII. Conclusion

In view of the foregoing, it is respectfully submitted that claims 1, 3-8, 10-24 and 44-46 are in condition for allowance and the application should be allowed in its present form. In particular, it is respectfully submitted that the presently pending rejections of claims 1, 3-8, 10-24 and 44-46 are improper and should be reversed for reasons as discussed above. In that regard, each of claims 1, 3-8, 10-24 and 44-46 is in condition for allowance.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 19-0741. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 19-0741. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 19-0741.

Respectfully submitted,

Date: September 23, 2008

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VIII. Claims Appendix

1. (Previously Presented) A method for formulating an enzyme comprising:

obtaining a library of glucose oxidase genes;

creating a library of mutated glucose oxidase genes;

introducing each mutated glucose oxidase gene of the library into separate expression vectors;

inserting the expression vectors into non-human host organisms;

growing colonies of the host organisms; and

screening the colonies for predefined, desired properties by determining whether the colonies contain active glucose oxidase and determining whether the colonies have predefined, desired peroxide resistant properties,

wherein determining whether the colonies have predefined, desired peroxide resistant properties comprises:

incubating the colonies in peroxide; and

determining whether the colonies have active glucose oxidase after incubating the colonies in peroxide.

2. Cancelled.

3. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein screening the colonies for desirable properties further comprises testing glucose oxidase from the colonies for functionality to function as a sensor enzyme in a sensor.

4. (Original) A method for formulating an enzyme according to claim 1, wherein determining whether the colonies have peroxide resistant properties is only performed if results of determining whether the colonies contain active glucose oxidase are positive.

5. (Original) A method for formulating an enzyme according to claim 3, wherein testing glucose oxidase from the colonies for functionality is only performed if results of determining whether the colonies contain active glucose oxidase are positive and if results of determining whether the colonies have peroxide resistant properties are positive.

6. (Previously presented) A method for formulating an enzyme according to claim 1, wherein determining whether the colonies have active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase.

7. (Original) A method for formulating an enzyme according to claim 6, wherein the substance is leuco-crystal-violet.

8. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein determining whether the colonies have active glucose oxidase comprises checking for fluorescence.

9. Cancelled.

10. (Previously Presented) A method for formulating an enzyme according to claim 3, wherein testing glucose oxidase from the colonies for functionality comprises employing glucose oxidase from the colonies in sensors.

11. (Previously Presented) A method for formulating an enzyme according to claim 10, wherein employing glucose oxidase from the colonies in sensors comprises:

extracting glucose oxidase from the colonies;

immobilizing the glucose oxidase after extracting the glucose oxidase from the colonies;

placing the immobilized glucose oxidase in a sensor; and

testing the sensor for functionality in a test environment.

12. (Original) A method for formulating an enzyme according to claim 11, wherein extracting glucose oxidase from the colonies comprises employing an ionic column to extract glucose oxidase from the colonies.

13. (Original) A method for formulating an enzyme according to claim 11, wherein extracting glucose oxidase from the colonies comprises:

removing the glucose oxidase from the colonies;

purifying the glucose oxidase; and

characterizing the glucose oxidase.

14. (Original) A method for formulating an enzyme according to claim 13, wherein removing the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell components.

15. (Original) A method for formulating an enzyme according to claim 14, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

16. (Original) A method for formulating an enzyme according to claim 13, wherein removing the glucose oxidase from the colonies comprises disrupting the colonies into cell components via sonication.

17. (Original) A method for formulating an enzyme according to claim 16, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after disrupting the colonies via sonication.

18. (Original) A method for formulating an enzyme according to claim 13, wherein purifying the glucose oxidase comprises purifying the glucose oxidase by employing chromatography methods.

19. (Previously Presented) A method for formulating an enzyme according to claim 1, wherein the glucose oxidase is obtained from an organism and wherein the organism is selected from a group consisting of *Aspergillus Niger, Penicillium funiculosum, Saccharomyces cerevisiae, and Escherichia Coli.*

20. (Original) A method for formulating an enzyme according to claim 1, wherein creating at least one mutated glucose oxidase gene comprises employing polymerase chain reaction techniques to create at least one mutated glucose oxidase gene.

21. (Original) A method for formulating an enzyme according to claim 1, wherein creating at least one mutated glucose oxidase gene comprises employing error-prone polymerase chain reaction techniques to create at least one mutated glucose oxidase gene.

22. (Original) A method for formulating an enzyme according to claim 1, wherein creating at least one mutated glucose oxidase gene comprises employing gene shuffling techniques to create at least one mutated glucose oxidase gene.

23. (Original) A method for formulating an enzyme according to claim 1, wherein the method further comprises creating a next generation of mutated glucose oxidase genes after screening the colonies for desirable properties.

24. (Original) A method for formulating an enzyme according to claim 23, wherein creating a next generation of mutated glucose oxidase genes is repeated approximately 2 to 6 times.

25. (Withdrawn) An enzyme formulated according to the method of claim 1.

26. (Withdrawn) A method for formulating an enzyme comprising:

obtaining an organism with a glucose oxidase gene;

growing multiple colonies of the organism;

altering the environment of the colonies; and

screening the colonies to identify colonies with active glucose oxidase after altering the environment of the colonies.

27. (Withdrawn) A method for formulating an enzyme according to claim 26, wherein the organism is selected from a group consisting of Aspergillus Niger, Penecillium funiculosum, Saccharomyces cerevisiae, and Escherichia Coli.

28. (Withdrawn) A method for formulating an enzyme according to claim 26, wherein altering the environment of the colonies comprises introducing peroxide to the colonies.

29. (Withdrawn) A method for formulating an enzyme according to claim 26, wherein screening the colonies to identify colonies with active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase.

30. (Withdrawn) A method for formulating an enzyme according to claim 29, wherein the substance is leuco-crystal-violet.

31. (Withdrawn) A method for formulating an enzyme according to claim 30, wherein screening the colonies to identify colonies with active glucose oxidase comprises checking for fluorescence.

32. (Withdrawn) A method for formulating an enzyme according to claim 26, wherein the method further comprises testing the colonies with active glucose oxidase for functionality after screening the colonies to identify colonies with active glucose oxidase.

33. (Withdrawn) A method for formulating an enzyme according to claim 32, wherein the method further comprises continuing to alter the environments of the colonies until the colonies with active glucose oxidase are of a suitable number to proceed with testing the colonies with active glucose oxidase for functionality.

34. (Withdrawn) A method for formulating an enzyme according to claim 32, wherein testing the colonies with active glucose oxidase for functionality comprises employing glucose oxidase from the colonies in sensors.

35. (Withdrawn) A method for formulating an enzyme according to claim 32, wherein testing the colonies with active glucose oxidase for functionality comprises:

extracting glucose oxidase from the colonies;

immobilizing the glucose oxidase after extracting the glucose oxidase from the colonies;

placing the immobilized glucose oxidase in a sensor; and

testing the sensor.

36. (Withdrawn) A method for formulating an enzyme according to claim 35, wherein extracting glucose oxidase from the colonies comprises employing an ionic column to extract glucose oxidase from the colonies.

37. (Withdrawn) A method for formulating an enzyme according to claim 35, wherein extracting glucose oxidase from the colonies comprises:

removing the glucose oxidase from the colonies;
purifying the glucose oxidase; and
characterizing the glucose oxidase.

38. (Withdrawn) A method for formulating an enzyme according to claim 37, wherein removing the glucose oxidase from the colonies comprises grinding the colonies in a homogenizer into cell components.

39. (Withdrawn) A method for formulating an enzyme according to claim 38, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after grinding the colonies in a homogenizer.

40. (Withdrawn) A method for formulating an enzyme according to claim 37, wherein removing the glucose oxidase from the colonies comprises disrupting the colonies into cell components via sonication.

41. (Withdrawn) A method for formulating an enzyme according to claim 40, wherein removing the glucose oxidase from the colonies further comprises fractionating the cell components employing centrifugation and differential solubility after disrupting the colonies via sonication.

42. (Withdrawn) A method for formulating an enzyme according to claim 37, wherein purifying the glucose oxidase comprises purifying the glucose oxidase by employing chromatography methods.

43. (Withdrawn) An enzyme formulated according to the method of claim 26.

44. (Previously Presented) The method of formulating an enzyme according to claim 1, wherein the host organism comprises a microorganism.

45. (Original) The method of formulating an enzyme according to claim 1, wherein determining whether the colonies contain active glucose oxidase further comprises isolating the glucose oxidase.

46. (Previously Presented) The method of formulating an enzyme according to claim 1, wherein screening the colonies for desirable properties further comprises:

isolating the glucose oxidase;

placing the glucose oxidase in a sensor; and

testing the sensor for functionality in a test environment.

47. (Cancelled)

48. (Withdrawn) A method for making a biosensor comprising:

obtaining a library of glucose oxidase genes;

creating a library of mutated glucose oxidase genes;

introducing each mutated glucose oxidase gene of the library into separate expression vectors;

inserting the expression vectors into a host;

growing colonies of the host;

screening the colonies for desirable properties by determining whether the colonies contain active glucose oxidase and determining whether the colonies have peroxide resistant properties; and

placing a glucose oxidase gene having desirable properties into a sensor,

wherein determining whether the colonies have peroxide resistant properties comprises:

incubating the colonies in peroxide; and

determining whether the colonies have active glucose oxidase after incubating the colonies in peroxide, and

wherein determining whether the colonies contain active glucose oxidase comprises:

measuring a concentration of the glucose oxidase.

49. (Withdrawn) A method for making a biosensor according to claim 47, wherein screening the colonies for desirable properties further comprises testing glucose oxidase from the colonies for functionality.

50. (Withdrawn) A method for making a biosensor according to claim 47, wherein determining whether the colonies have active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase.

51. (Withdrawn) A method for making a biosensor according to claim 47, wherein determining whether the colonies have active glucose oxidase comprises employing a substance that changes color in the presence of active glucose oxidase.

52. (Withdrawn) A method for making a biosensor according to claim 51, wherein the substance is leuco-crystal-violet.

53. (Withdrawn) A method for making a biosensor according to claim 47, wherein determining whether the colonies have active glucose oxidase comprises checking for fluorescence.

54. (Withdrawn) A method for making a biosensor according to claim 47, wherein the host is a host organism.

IX. Evidence Appendix

None.

X. Related Proceedings Appendix

None.